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Dear Dave:

The purpose of this is to give you a better idea of what is going on in the lab, but without writing a book. The "figures" to which I refer are hand drawn on the attached sheets.

There are two major threads in the work at present, as well as one matter still in the beginning stage. I will not describe the latter, but you may want to talk with Ron Thayer about the work he is doing concerning monkey sequences that recombine (in vivo) with SV40. Ron is, in theory, my technician, but in fact functions like a very senior post-doc. Also, it is Ron who will be helpful in hundreds of ways, both about procedures, procuring chemicals and equipment, and the whereabouts of everything and everybody.

By the way, I have in addition to the big fancy office of the Laboratory Chief, a tiny closet in the lab (4A-01). Since I will be away a fair amount, I usually only use it afternoons anyway, and nothing else is available, you are to make yourself at home there. It is approximately two meters by three meters in size, and also accommodates our light box, our refractometer, various equipment we keep hidden from the vultures on the corridor, a black board, and often four people talking.

First major project: detection, isolation and characterization of monkey DNA sequences that have homology to the control regions (non-transcribed) around the origin of replication of SV40. Three lambda phage were picked out of a monkey "library" by virtue of such homology. One of these is described in the preprint by McCutchan, which I sent. Tom McCutchan has now left the lab, and this project is being carried forward by Susan Lord and Cary Queen. The sequences of the SV40like regions in each of the three phage have been obtained. All three contain the same funny scrambled mixture of SV40-like pieces found in Toni's phage. Also, genomic blots indicate that while only three phage were found that hybridized with SV40, sequences of this type are abundant. We guess at about 100 copies, but do not know. We would like to know and this requires some hybridization kinetics, thus these sequences are one candidate for cloning in a system that will afford single stranded probes (like M13, see attached). Some work aimed at trying to determine the function of these monkey sequences is going on in collaboration with Carol Prives at Columbia (T-antigen binding) and Paul Berg (testing the possibility that the sequences function as promotors).

The second major project: analysis of the structure and organization of a highly repeated 172 base pair segment of monkey DNA. In toto, probably 5×10^6 copies of this exist and the whole together is called alpha-component (see attached reprint with the flashy title). I think I sent preprint by Thayer, McCutchan and Singer which has some recent information on this. For some rather complex reasons, the monkey library described about afforded us a large number of phage which contain alpha-component sequences but also non-alpha. That is, they contain junctions between the highly repeated sequence and other genomic segments. We are trying to study these other segments. Of about 17 phage, several fall into two classes defined by common neighboring sequences. Class 1 (Fig. 1) contains alpha sequence, then a fragment of a particular size, and then a segment with sequences homologous to another repeated class of DNA called "alu". Finally, on the left, a variety of things. Class two members have in common a 1 kb fragment produced by endonuclease BamH1. In one case we know it is very close to the alpha sequences, as shown in the Figure. The other members of this class are being mapped. In each instance we will need to subclone the common non-alpha "junction" sequences and ask about their reiteration frequency in the monkey genome as well as whether they occur only next to alpha or in other places as well. Again, we will need to put these subcloned fragments into a cloning system that affords single stranded probes.

Thus for a variety of reasons it would be helpful to set up a system for making single stranded probes. There are two available. One is the M13. You can see from the attached that all the reagents can be obtained easily. The M13 system's main advantage is the preparation of fragments for sequencing. The disadvantage for preparing probes is that the vector itself is about 6000 base pairs long and therefore the cloned segment will only represent a small proportion of the total. An alternative is to use a pBR322 derivative developed by Marty Rosenberg and his colleagues here. This vector has an active E. coli promotor placed just before the sight at which a foreign DNA fragment is cloned. Such a recombinant can be prepared in good yield as a plasmid and a probe prepared by transcribing the vector in vitro. By cutting the vector at a built in site beyond the foreign insert, the transcription will stop shortly after. Thus the probe (RNA, not DNA now) will contain only a few hundred extraneous bases. Marty uses this vector (Fig. 2) to study transcription regulation in coli, so this would be a new use for it. The RNA probe can be purified by a single gel electrophoresis.

I favor the Rosenberg system over the M13 for our present purposes primarily because the job of purifying the desired probe away from extraneous material will be much simpler. People in Marty's lab will be helpful in getting things going and some of them are actually in with us for now while Marty awaits some renovations.

In fact though, some of the structural work and subcloning on the alpha-containing phage may not be done before you arrive. In that case, I would suggest you go to work helping out on those projects. Antonella Maresca (an Italian post-doc) is working on the 1 kb Bam fragment. But the most likely thing is that Giovanna Grimaldi (another Italian post-doc) will be happy to have someone help with the fragment that is between alu and alpha. She is simultaneously trying to do something else and would welcome a colleague. She is very good at all the techniques and what she doesn't know, Ron or the others can help with. I suggest that you "report" to Giovanna to begin with. If she has finished the subcloning, then you could get to work on the Rosenberg system. I will grease all the wheels before I leave on vacation (December 23).

The only lab person to whom I have not "introduced" you is Theresa Lee. Theresa is working on the isolation of alpha-component sequences from a single monkey chromosome, which she isolated by fusion of monkey cells with TK^- mouse cells and selection for TK^+ in HAT. After some months of passaging she has cloned with a single monkey chromosome (by staining) and is now making a library from such DNA, to be screened for the monkey sequence.

Two more people whose names you should have. Ms. Gloria Tatigian serves as my administrative assistant in running the lab and can be found in the lab office (4E-28). Also in that room is Ms. May Liu who serves as secretary to our Section. I expect that both of them can be quite helpful with a variety of matters. In my absence Ed Kuff is acting laboratory chief.

Welcome.

Sincerely,

Maxine Singer

Encl.